Gibson Assembly

Aim

To assemble two or more DNA fragments into a vector using Gibson Assembly.

Procedure

1. Set up the following components on ice.

Table 1: Reaction Components for 2-3 Fragment Assembly

Component	Volume [μl]
DNA Fragments (0.02-0.05 pmols)	X
Gibson Assembly Master Mix (2X)	10
dH_2O	10-X
Total volume	20

   For optimal cloning efficiency, use 50-100 ng of vector with 2- folds of excess inserts. If fragment is below 200 bp use 5-fold excess of insert.

2. Incubate inside a thermocycler at 50 °C for 15 minutes when 2-3 fragments are being assembled.

3. Store sample on ice or at -20 °C for transformation.

Note!

Ensure that the fragments contain overlapping regions of 15-40 bp that exhibit a melting temperature larger than 48 °C.

Sources

This protocol is provided by the Assembly Master Mix - Assembly (E2611) kit from New England Biolabs. The link to this protocol can also be found at the address below.
https://www.protocols.io/view/Gibson-Assembly-Master-Mix-Assembly-E2611-imsupm